

(d) a second structural gene expressible in eukaryotic cells, located 3' to said IRES such that the second structural gene is placed under the translational control of said IRES, such that the first structural gene, IRES and the second structural gene are transcribed under the action of said transcriptional promoter to give a primary transcript, wherein the first structural gene of the primary transcript is able to translate by ribosome scanning mechanism and the translation of the second structural gene of the primary transcript is mediated by said IRES.

20. A recombinant nucleic acid sequence comprising:

- (a) a transcriptional promoter;
- (b) a first structural gene expressible in eukaryotic cells linked to said transcriptional promoter;
- (c) a nucleic acid sequence functional as an internal ribosome entry site (IRES) characterized by being
  - (i) of plant viral origin;
  - (ii) located upstream of a 5' distal structural gene;
  - (iii) purine-rich and/or having a stem loop structure;and
  - (iv) capable of promoting cap-independent expression of 5'-distal genes in eukaryotic cells from bicistronic and/or polycistronic mRNAs;

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(d) a second structural gene expressible in eukaryotic cells, located 3' to said IRES such that the second structural gene is placed under the translational control of said IRES, such that the first structural gene, IRES and the second structural gene are transcribed under the action of said transcriptional promoter to give a primary transcript, wherein the first structural gene of the primary transcript is able to translate by ribosome scanning mechanism and the translation of the second structural gene of the primary transcript is mediated by said IRES.

Spec 21. A recombinant nucleic acid sequence comprising:

- (a) a transcriptional promoter;
- (b) a first structural gene expressible in eukaryotic cells linked to said transcriptional promoter;
- (c) a nucleic acid sequence of plant viral origin designated as an internal ribosome entry site (IRES) by promoting cap-independent expression of a 5'-distal gene in eukaryotic cells from bicistronic and/or polycistronic mRNAs, whereby said nucleic acid sequence is designated by utilizing crTMV RNA sequences upstream of the MP gene or the CP gene;
- (d) a second structural gene expressible in eukaryotic cells, located 3' to said IRES such that the second structural gene is placed under the translational control of said IRES, such that the first structural gene, IRES and the second structural gene are

transcribed under the action of said transcriptional promoter to give a primary transcript, wherein the first structural gene of the primary transcript is able to translate by ribosome scanning mechanism and the translation of the second structural gene of the primary transcript is mediated by said IRES.

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22. The recombinant nucleic acid sequence according to claim 21, wherein said nucleic acid sequence of (c) is further characterized by being purine-rich and/or having a stem loop structure.

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23. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein said IRES is a nucleic acid sequence upstream of the movement protein gene of a plant virus belonging to the group of tobamoviruses and exhibiting IRES-activity, i.e. being capable of promoting expression of the 5'-distal genes from bicistronic and/or polycistronic mRNAs in eukaryotic cells.

24. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein said IRES is a nucleic acid sequence upstream of the coat protein gene of a plant virus belonging to the group of tobamoviruses and exhibiting IRES activity, i.e. being capable of promoting expression of the 5'-distal genes from bicistronic and/or polycistronic mRNA in eukaryotic cells.

25. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein said IRES is capable of promoting expression of 5'-distal gene(s) from bicistronic and/or polycistronic constructs in plant cell cultures and/or transgenic plants.

B<sup>1</sup> 26. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein said IRES is capable of promoting expression of 5'-distal gene(s) from bicistronic and/or polycistronic constructs in human and animal cell cultures and/or transgenic animals.

27. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein said IRES is capable of promoting expression of 5'-distal gene(s) from bicistronic and/or polycistronic constructs in transformed yeast cells.

28. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein said IRES sequences are used *in vitro* in cell-free protein synthesizing systems.

29. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein at least one of the structural genes encodes a desired polypeptide product selected from the group consisting of

selectable markers, toxins, hormones, gene silencing suppressing proteins, proteases or viral proteins.

30. The recombinant nucleic acid sequence according to claim 29, wherein said structural gene encoding the selectable marker confers antibiotic resistance, herbicide resistance, color change, or encodes a polypeptide which is capable of reacting with a compound to produce a detectable signal.

31. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein the transcriptional promoter is a constitutive or inducible eukaryotic-specific promoter.

32. The recombinant nucleic acid sequence according to claim 31, wherein the inducible promoter is activated by environmental conditions, by pathogens, or by chemicals aimed for plant, yeast, animal or human protection.

33. The recombinant nucleic acid sequence according to claim 19, 20 or 21, wherein the nucleotide sequence additionally comprises at 3'-position of said second structural gene an IRES, which may be the same or different, and an additional downstream gene encoding a desired polypeptide product to give a polycistronic mRNA.

34. The recombinant nucleic acid sequence according to claim 33, wherein said polycistronic mRNA provides for coordinated expression of multiple polypeptides or several enzymes of a biosynthetic pathway.

*Sub C2* 35. A method for coexpressing two or more genes producing two or more proteins or polypeptides of interest in eukaryotic cells, comprising introducing into said cells a recombinant nucleic acid sequence according to claim 19.

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*canal* 36. A eukaryotic cell transformed with a recombinant nucleic acid sequence according to claim 19, 20 or 21.

37. The eukaryotic cell according to claim 36, wherein the cell is selected from the group consisting of yeast cells, plant cells, insect cells and mammalian cells.

38. A transgenic eukaryotic organism transformed with a recombinant nucleic acid sequence according to claim 19, 20 or 21.

39. The transgenic eukaryotic organism according to claim 38, wherein the organism is selected from the group consisting of plants and animals.